

• **Use of stomach tubing as an alternative to rumen cannulation to study the bacterial community in sheep and goats**

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Rumen cannulation is considered the reference method for collection of representative samples of rumen digesta and is widely used in ruminant nutrition research. However, it is not feasible in lactating ewes or goats, because of adverse effects on animal performance, which obliges to depend on less invasive techniques, such as stomach tubing. The aim of this work was to study whether collecting samples through cannula or stomach tube detect the differences in the rumen bacterial community between species (sheep and goats) and diets (forage and forage plus concentrate, 1:1). Four sheep and four goats, fitted with ruminal cannula, were fed either forage or forage plus concentrate, in two 15-d periods. At the end of each period, samples of rumen digesta were taken after feeding, first by stomach tube and immediately afterwards via rumen cannula. PCR-DGGE analysis of the bacterial community was carried out.

The similarity dendrogram showed that, within period, animal species was the factor driving the grouping pattern, which was more evident in samples collected through cannula. Furthermore, diversity indexes were higher for goats than for sheep only when sampling via cannula. None of the techniques detected significant variations in the bacterial diversity when comparing the diets. Our results show that stomach tubing allows the collection of a highly diverse bacterial community that is able to detect some of the effects observed when sampling through cannula. However, further studies in other microbial groups, using high-throughput sequencing tools, are needed to explore differences in the abundances of some microbial taxa.

**A novel co-culture system showed beneficial interaction between human intestinal epithelial cells and the anti-inflammatory anaerobic gut bacterium *Faecalibacterium prausnitzii*.**

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*Faecalibacterium prausnitzii* is one of the most important suppliers of the butyrate in the gut ecosystem. Reduction in the abundance of this bacterium is correlated with the higher risk of Crohn's disease. The extreme oxygen sensitivity of this bacterium has been restrained the in depth investigation of potentially beneficial properties of living *F. prausnitzii* on intestinal health in vitro. Here we promote a simple co-culture method to study the interaction between the *F. prausnitzii* and caco-2 cell line. *F. prausnitzii* A2-165 pure cultures were inoculated in 50ml tubes containing YCFAG-agar medium. Caco-2 cells were mounted on top of such a culture and tubes were filled with DMEM medium. The growth pattern of the *F. prausnitzii* was investigated by visualizing its growth rim. The cytokine profiles of caco-2 cell were measured. The amounts of short chain fatty acids in DMEM medium were determined by Chromatography. Liquid Chromatography-Mass Spectrometry was used for metabolomics analysis of the DMEM medium.

In co-culture system, Caco-2 cells were capable of stimulating the growth of *F. prausnitzii* outside of the anaerobic chamber. *Faecalibacterial* cells were formed thicker growth rims in oxygenated zone of the YCFAG-agar in co-cultures compared to the controls. Production of SCFAs in the co-cultures was different of the ones of the controls. In addition *F. prausnitzii* suppresses the expression of inflammatory and oxidative-stress markers in Caco-2 cells.

In this model faecalibacterial cells were capable of growing outside of the anaerobic chamber and promoted anti-inflammatory and anti-stress effects on Caco-2 cells and consecutively their growth were stimulated by Caco-2 cells.

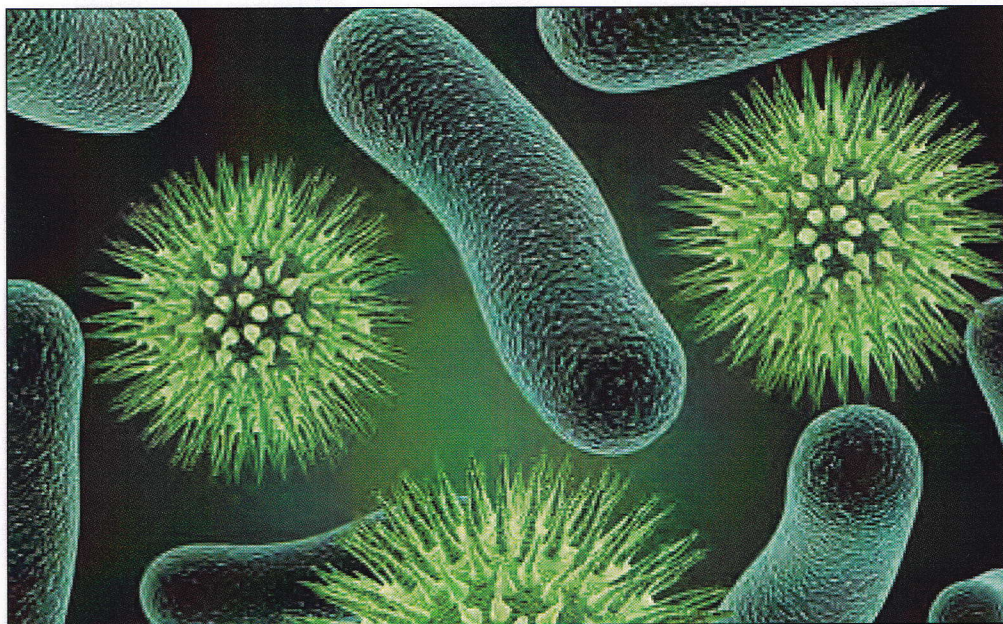


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